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09/966,522	09/28/2001	Thomas Krahn	100717-502 / Bayer 10139	5606

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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

ADVISORY ACTION

Priority

Parent Data

This application, 09966522, filed 09/28/2001 (PGPub **20020009754)** and having 1 RCE-type filing therein and is a continuation of 09194099, filed 11/20/1998 ,now U.S. Patent #6420183 and having 2 RCE-type filings therein. Application 09194099 is a national stage entry of PCT/EP97/02662 , International Filing Date: 05/23/1997, and claims foreign priority to 19621312.6 , filed 05/28/1996.

Child Data

Application 10263607, filed on 10/03/2002 ,now U.S. Patent #7138280 and having 1 RCE-type filing therein, is a division of 09966522 , filed on 09/28/2001 and having 1 RCE-type filing therein. Application 12199317 (just allowed), filed on 08/27/2008 is a division of 09966522 , filed on 09/28/2001 and having 1 RCE-type filing therein.

Amendment Entry & Claims Status

The request for reconsideration after a final rejection filed on November 23, 2009 has been acknowledged and entered.

Claims 17-23 and 43 are pending and being examined.

Claims 6-16, 24-42 are withdrawn from further consideration due to a non-elected invention.

Maintained Rejection(s)

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 17-21, 23 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Roth et al. (US 5,545,535 filed on November 1, 1993) in view of Wan et al. (Journal of Immunological Methods 162 (1993) pp. 1-7) and further in view of Cubbage (US 5,582,982).

Roth teaches using four different fluorescent dyes to label bacteria. One of the dyes is a fluorescent dye that is highly membrane-permeant cyanide dye derivatives and labels all bacteria and stains the nucleic acid of the bacterial cell. (see abstract, dye formula I; col. 6, line 55-col. 13, line 28).

However, Roth fails to teach a masking dye in a solution to reduce non-specific background light emitted from said solution by at least 10%, 30%, 50% or 70% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

Wan teaches a method of using fluorescein conjugated E.Coli particles and second dye such as Trypan blue to quench the extracellular fluorescence in the solution. That means Trypan blue absorbs and the extracellular fluorescence which cause the solution to emit non-specific background light in the solution while the fluorescent that absorbs into the cells are being measured. Quenching the extracellular fluorescence thus means reducing non-specific background light in solution. (see abstract, page 3 “Phagocytosis assay” and “results”). Trypan blue is obviously impermeant to the membrane of the cell because it quenches extracellular fluorescence. Wan also teaches that the concentration of trypan blue require to completely

quench extracellular fluorescence was determined by exposing 3 or 6 $x10^8$ particles/well to serial dilutions of the dye in a 96-well plate. Complete quenching of the fluorescence was obtained with 250 ug/ml of the dye. Thus, Wan meets the requirement that the non-specific background in solution is reduced by at least 30%, 50% and 70% (claims 18-20). Since trypan blue can quench or reduce non-specific background, it would be able to perform functions such as to improve the signal to noise ratio by at least 300%.

It would have been obvious to one of ordinary skills in the art to use an extra dye such Trypan blue as taught in Wan to quench extracellular fluorescence in the method of Roth because Roth uses a combination of four fluorescent dyes to stain cells and thus there would be plenty of extracellular fluorescence which would cause non-specific background light, and Trypan blue can completely quench extracellular fluorescence.

However, Roth and Wan fail to teach a kit.

Cubbage teaches a kit comprising a fluorescent probe and a background-reducing compound that diffuses into and onto the biological entity. (see col. 2, line 45-col. 7, line 27).

It would have been obvious to one of ordinary skills in the art package the components taught by Roth and Wan into a kit as taught by Cubbage to the advantage of economical convenience and storage.

Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over Roth in view of Wan et al. (Journal of Immunological Methods 162 (1993) pp. 1-7), and further in view of Cubbage et al. (US 5,582,982) as applied to claim 17-21, 23 above, and further in view of Van Aken (US 5,489,537).

Roth, Wan and Cubbage have been discussed above.

However, Roth, Wan and Cubbage fails to teach Brilliant Black as a fluorescent dye.

Van Aken teaches a method and kit for determining the presence or absence of a substance by detection of a colloidal dye associated with agglutinated particles. The colloidal dye is a background-enhancing dye, which reduces non-specific background to enhance optical detection. The background-enhancing dye is a water-soluble dye such as Brilliant Black. (see col. 21, lines 58-67).

It would have been obvious to one of ordinary skills in the art to use Brilliant Black as a masking or quenching dye in the kit for use in the method of Roth, Wan and Cubbage because these references teach using quenching or background reducing dye, which reduces background light in assay. Since Brilliant Black is known for enhancing the background in an assay, which uses optical detection, it would motivate one of ordinary skills in the art to use Brilliant Black in assays such as one taught by Wan and Cubbage because both Wan and Cubbage teach using fluorescent label, which is known for producing non-specific background.

Response to Arguments

Applicant's arguments filed November 23, 2009 have been fully considered but they are not persuasive.

Regarding the 103 rejections, Applicants argue that because Roth (US 5,545,535) focused on the detection of dead cells or membrane-compromised cells and Roth describes the fluorescent dyes of formula I to IV with respect to their impact on dead bacteria or on cells with compromised plasma membrane integrity, a person skilled in the art would not have been motivated to add trypan blue or any other masking dye to Roth's assay. Such masking dye would

have been expected not only to stain the ambience of the cells and to reduce background fluorescence but would also have been expect to permeate into the dead cells and also to quench signals from these cells. Such state of affairs would render Roth's assay unworkable and the results would be inaccurate.

This is not found persuasive because the abstract of Roth describes "A method for analyzing a sample thought to contain bacteria using a total of four dyes which stains all viable and dead cells". Particularly, dye formula I stains all dead or viable cells. Thus, Roth does not focus on the detection of just dead or membrane-compromised cells but also viable cells. Therefore, there is a need to quench the extracellular fluorescence of the viable cells and thus combining Trypan blue would be obvious to one of ordinary skills in the art. Trypan blue might quench the signal from dead cells but Roth detects viable cells as well. The dye formula II and formula IV detects the viable cells and outer surface of all the cells. Thus, Trypan blue can quench extracellular fluorescence of at least the dye of formula IV.

Applicants also discussed the toxicity of Trypan blue on page 5 of response and argue that Trypan blue would convert viable cells into additional dead cells and therefore one of ordinary skills in the art would not add trypan blue to Roth's method.

This is not found persuasive because Trypan blue is known in the art to stain dead cells to distinguish from the viable cells. (see Friedman US 3,785,735, col. 1, lines 40-62). If Trypan blue causes viable cells to become dead cells, then it would not have been used to count dead cells and viable cells in a sample as taught by Friedman.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Pensee T. Do whose telephone number is 571-272-0819. The examiner can normally be reached on Monday-Friday, 9-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya can be reached on 571-272-0806. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Pensee T. Do/
Examiner, Art Unit 1641

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